SHORT PAPER

Modifying mutations of the specific activity of alcohol dehydrogenase caused by a putative transposon in *Drosophila melanogaster*

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ABSTRACT

An extremely large detrimental to lethal load ratio (D/L ratio) of homozygous viability was detected in the Osaka natural population of *Drosophila melanogaster* in 1979. Mutations were accumulated on 79 second chromosome lines that originated from one of the Osaka isogenic second chromosome lines. After five generations the genetic load and ADH specific activity were measured. The results suggest that a putative transposon which is tentatively called "MY factor" induced an extremely high frequency of mutations most probably in non-coding regions, and that some of these mutations modify the ADH specific activity.

1. INTRODUCTION

In *Drosophila melanogaster* populations, many types of movable genetic elements such as the P, I, copia, and FB elements have been detected (Kidwell *et al.* 1977; Picard 1976; Finnegan *et al.* 1978; and Potter *et al.* 1980). It is an important problem to clarify whether these elements or transposons are "selfish DNA" or play some significant role in adaptive microevolution.

Mukai, Harada and Yoshimaru (1984) suggested that insertion or excision of some type of transposon in non-coding regions modifies the specific activity of alcohol dehydrogenase (ADH). Extremely high detrimental to lethal load ratio (D/L ratio) concerning viability (cf. Greenberg and Crow 1960) was observed in the second chromosomes of the Osaka natural population of *Drosophila melanogaster* in 1979 (Mukai and Yukuhiro 1983). Mukai and Cockerham (1977) and Mukai, Harada and Yoshimaru (1984) statistically suggested that most of the mildly deleterious mutations occur outside the structural loci or in non-coding regions.

Using one of the second chromosomes extracted from the Osaka population, a mutation accumulation experiment was conducted. The results indicate

that the mildly deleterious mutation rate was more than ten times higher than the previously estimated values, but that the lethal mutation rate is only twice that of the previous estimates (cf. Mukai 1964; Mukai *et al.* 1972). In addition, male recombination and unique inversions occurred in the Osaka isogenic lines. Thus, it was speculated that there is some type of transposon which is different from the members of the P element family (Iwano *et al.* 1984) and tentatively called "MY factor" (Yukuhiro and Mukai 1984), and that by the insertion or excision of this putative transposon to or from the non-coding regions, mildly deleterious mutations occurred. If this is the case, these mildly deleterious mutations might modify the specific activity of the enzymes produced by structural genes. Thus, using the above-mentioned mutation accumulation lines derived from a single second chromosome, the specific activities of alcohol dehydrogenase (ADH) were measured. The result is reported in this short paper.

2. MATERIALS AND METHODS

The original isofemale lines were established using females collected at Komagatani, Osaka, in October, 1978 by Dr. S. I. Chigusa, Ochanomizu University. One of the "isogenic" second chromosome lines (WY-113) was chosen since a unique inversion was segregating in this originally isogenic line and spontaneous mutations were accumulated for five generations in 79 lines that originated from a single second chromosome. The viability was tested by the *Cy* method (cf. Wallace 1956). Details of the methods are described in the previous paper (Mukai and Yukuhiro 1983). For the measurement of the alcohol dehydrogenase (ADH) specific activity, crosses were made between ten *Cy*/+ males and ten *Cy*/+ females for the identical +chromosomes. Six to twelve vials of the crosses were made for each line. Emerged flies were collected and pooled in each line. They were aged for four days and fifteen +/+ males were randomly sampled. They were randomly divided into three groups of five males each and used for the ADH activity assay. The methods of ADH activity and protein assays are described elsewhere (see Mukai, Harada and Yoshimaru 1984). A total of 43 lines were employed. One of the mutation accumulation lines (WY-113-8-2) which showed the highest viability among these lines was chosen for the control. This line was expanded to the same number of lines as the mutation accumulation lines. The control lines were cultured at the same time as the mutation accumulation lines and were used for the ADH activity assay after randomization together with the mutation accumulation lines.

3. RESULTS AND DISCUSSION

The activity assay for ADH was done successively at four different times. The numbers of lines employed were ten, twelve, fifteen and six in both the

Fig. 1. Frequency distribution of lines with respect to specific activity of ADH. Mutations were accumulated for 5 generations.

Table 1. *Results of analysis of variance for the ADH specific activity*

	Control	Accumulated
Mean	0.2882	0.2974
(U/mg)	± 0.0021	± 0.0031
Genetic Variance	-0.6196×10^{-4}	0.2566×10^{-3}
Component (U^2/mg^2)	$\pm 0.6513 \times 10^{-4}$	$\pm 0.1086 \times 10^{-3}$

experimental and the control groups. The distribution pattern of the ADH specific activities after standardization by assuming the average specific activities of the control to be 1.0 is illustrated in Figure 1. It is noted that the activities spread over both directions. The mean for the mutation-accumulation lines is higher than that of the control. This may imply that the average effect of the mutations on the ADH specific activity was increased or that the control line had acquired mutations before the expansion which decreased ADH specific activity. One-way analysis of variance was done for each group of the activity assay and the mean squares were calculated from the pooled sum of squares (Table 1). The F test showed significant line effects at the 1% level in the mutation-accumulation lines ($F_{39,86}=2.26$) but not in the control experiment ($F_{39,86}=0.79$). Two exceptional lines (high and low) in the mutation-accumulation lines were excluded for a more strict analysis. The F value, however, was still significant at the 5% level ($F_{37,82}=1.70$).

Mukai and Yukuhiro (1983) estimated the mutation rate of viability polygenes for the present material to be 1.95 per second chromosome per generation. This extraordinarily high mutation rate can not be explained without considering the effect of mutators or transposons. Indeed, the mutations were accumulated in the M cytotype of the P-M dysgenic system which is considered to be deficient in the inhibitor of hypothetical transposase (O'Hare

and Rubin 1983). It is likely that insertion or excision of transposons affected viability and, at the same time, modified the enzyme activity. Assuming that all the viability mutations control ADH specific activity, the following relationship can be obtained from Mukai and Yukuhiro (1983):

$$M \leq \sqrt{V \sum_{i=1}^n \mu_i}$$

$$= \sqrt{1.95 \times 0.3684 \times 10^{-4}} = 0.085,$$

where μ_i indicates mutation rate per generation at the i -th locus and the summation is over all relevant loci (n) in the second chromosome. Then, $\sum_{i=1}^n \mu_i \geq 1.95$ (see Mukai and Yukuhiro 1983). M stands for the amount of change in mean specific activity per generation, and V is the magnitude of increase in genetic variance per generation among the second chromosome lines. The estimated activity change is larger than the observed value ($=0.0326 \pm 0.0115$). Alternative assumptions for this discrepancy are (1) that the mutations affecting the ADH activity occupy not all but only a limited fraction of the viability mutations and (2) that the direction of the effects of viability mutations is uni-directional but that of the effects on ADH specific activity is bi-directional. The correlation between the viability and the ADH activity was examined using 31 lines in which both measurements were made. The phenotypic correlation was -0.004 and is not statistically significant ($F_{1,29} = 5.69 \times 10^{-3}$). The genetic correlation is 0.095 ± 0.238 . Although the number of lines used for this analysis is small, there is no apparent correlation between ADH activity and viability. This result supports the alternative explanations. The possibility that the mutations of the ADH structural gene increased the genetic variance of the ADH activity is almost zero because the rate is very low. Actually no electromorph mutations at the ADH locus were observed in these lines by starch-gel electrophoresis. Most of the mildly deleterious genes (so-called mutant viability polygenes) are hypothesized, on the basis of statistical analysis, to be located outside the structural loci (Mukai and Cockerham 1977) and some of them are speculated to be controlling factors of structural genes (Mukai, Harada and Yoshimaru 1984). Therefore, the activity mutations as well as viability mutations are most plausibly caused by the insertion or excision of transposons in non-coding regions. Several eye color mutations are known to be induced by insertion or excision of transposons and some of them are associated with minor chromosome aberrations (for example in w^i mutant by the FB element. See Collins and Rubin 1982). It is also suggested that insertion of the copia elements into TATA box of the histone gene may change the rate of transcription (Ikenaga and Saigo 1982). The mutations induced by transposons in the present experiment may be different from the regulatory system located in the adjacent region of the structural genes, since

the mutation rate is extremely high. It is possible that induced minor chromosome aberrations or chromosome rearrangements in the non-coding regions in a broad sense change enzyme activities, or the inserted transposon itself may modify the activity of the structural genes and could become the source of adaptive microevolution. Our MY factor may be one of the candidate for such a transposon.

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